IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
NAKAMURA et al.	Art Unit: 1652
Application No.: 10/720,177	Examiner: Delia M. RAMIREZ
Filing Date: November 25, 2003	Attorney Ref. No.: US-110
For: METHOD FOR PRODUCING L-GLUTAMINE AND L-GLUTAMINE PRODUCING BACTERIUM	Confirmation No.: 6388

PRE-APPEAL BRIEF REQUEST FOR REVIEW

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Commissioner for Patents
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Sir:

In response to the Final Official Action dated March 17, 2008, the response period set to expire on June 17, 2008, Applicant requests a Pre-Appeal Brief Review in accordance with the guidelines set forth in the July 12, 2005 Official Gazette Notice. Reconsideration of this application by a three Examiner panel is requested in view of the following remarks which identify the errors in facts, and the omission of essential elements required to establish a *prima facie* rejection. A Notice of Appeal was previously filed on January 16, 2008.

Summary of Final Office Action and Status of Case

In the March 17, 2008 Office Action, claims 1, 4-5, 13-16, and 18-21 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Claims 1, 4-5, 12-16, and 18-19 were rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description and scope of enablement requirements. Claims 1, 4-5, 7, 13-16 and 19 were rejected under 35 U.S.C. §103(a) over EP 1229121 to Nakamura et al. (Nakamura'121) in view of Pompejus et al. WO 01/00843 (Pompejus'843) in further view of Duran et al..

Summary of Claimed Invention

The claimed invention is directed to an isolated coryneform bacterium having the ability to produce L-glutamine, wherein the bacterium has been modified by disrupting or mutating a glutaminase gene on the chromosome so that the glutaminase activity of the bacterium is reduced to 0.1 U/mg of cellular protein or less, wherein said glutaminase gene is selected from the group consisting of: a) a DNA comprising the DNA sequence of SEQ ID NO: 1, and b) a DNA which is able to hybridize with the DNA of SEQ ID NO: 1 under stringent conditions of 1 X SSC, 0.1% SDS, at 60°C, and is 95% or more homologous to SEQ ID NO: 1. Dependent claims,

particularly claim 12, recite that the glutaminase gene encodes a protein comprising the amino acid sequence of SEQ ID NO: 2.

In another embodiment, the claimed invention is directed to the bacterial cell as described above, but which is further modified by increasing the expression of a glutamine synthetase gene by increasing the copy number of said glutamine synthetase gene or by replacing a promoter region of the glutamine synthetase gene with a stronger promoter so that said glutamine synthetase activity of the bacterium is enhanced, wherein said glutamine synthetase gene is selected from the group consisting of: c) a DNA comprising the DNA sequence of SEQ ID NO: 3, and d) a DNA which is able to hybridize with the DNA of SEQ ID NO: 3 under stringent conditions of 1 X SSC, 0.1% SDS, at 60°C, and is 95% or more homologous to SEQ ID NO: 3, and which encodes a protein which has glutamine synthetase activity.

Factual Errors Requiring Review

Applicants appreciate the new Office Action which clarifies the status of claim 12. However, as explained below, the rejection under 35 U.S.C. §112, 1st paragraph seems entirely based upon the presence of the language "95% or more homologous to SEQ ID NO: 1", which is not present in claim 12. Therefore, it is a ***clear error*** that the basis for the rejection of claim 12 is not adequately explained.

A second clear error lies in the Examiner's refusal to grant any patentable weight to the claimed phrase "95% or more homologous to SEQ ID NO: 1". The Examiner cannot choose to ignore particular claim limitations. As several of the rejections rely on a correct interpretation of this phrase, the merits of this phrase and the patentable weight it lends to the claims is discussed at length.

In the rejection of this phrase under 35 U.S.C. §112, 2nd paragraph, the Examiner has stated that the phrase is "unclear and confusing in the absence of a definition providing the intended meaning of the term or the intended parameters required to determine the required homology value." Such ignores established precedent in Group 1600, and particularly precedent set by other Examiners and the Supervisor ***in the Examiner's own art unit.*** Specifically, Applicants have numerous patents issued from this art unit directed to similar-type claims, for example, bacteria which have been modified to increase or decrease expression of a known gene and its homologs within 90-95% homology for the purpose of producing a substance, usually an amino acid, wherein the supporting disclosure describing determination of homology is the same. Recent patents of Applicants issuing from art unit 1652 having homology language for defining similar genes or proteins in a claim include 7,300,786, 7,186,531, 7,160,704, and 6,893,852. Furthermore, the following patents have homology language in the claims, but were issued from a different group 1600 art unit, including one (the '998 patent) which was issued on an order from the Board of Patent Appeals and Interferences: 7,312,058, 7,244,569, 7,090,998, and 6,905,819. Therefore, it is clear that the use of homology language to define similar sequences in a claim is an accepted method for claiming variants to a claimed amino acid or gene sequence.

A major reason why such language is universally accepted as being clear and definite lies not so much in the patent application's specification, but in the standardized and well-practiced methods for determining homology between sequences known to those of skill in the art. It is well known in the art how to calculate homology using the computer program BLAST. The parameters used in this program, although they can be varied, they have been standardized, and so determination between sequence is commonly and routinely practiced in the art with consistent and meaningful results recognizable to those of skill in the art. Therefore, computer

program BLAST has standardized the methods for comparing sequences and establishing a homology, and is commonly and routinely used for such. For these reasons, the use of the homology language in the claims is clear and definite as one of ordinary skill in the art would be able to determine the sequences which fall within the 95% homology limitation to the recited SEQ ID NO. The rejection under 35 U.S.C. 112, 2nd should be withdrawn.

The Examiner has also made a rejection of the claims under 35 U.S.C. §112, 1st paragraph for both lack of adequate written description an lack of enablement. In responding to Applicants arguments and response submitted on July 31, 2007, the Examiner states that the phrase "95% or more homologous" has been given no weight, which represents a ***clear error***. If the Examiner was to give this phrase it's proper weight based on the clear and definite meaning known in the art as explained above, she would determine the claims are fully described and enabled. This is based upon evidence provided by Applicants with their response of July 31, 2007 in the form of sequence alignments showing the wealth of information known in the art about the claimed genes and proteins, and their highly similar homologs isolated and reported from other bacterial species and strains.

Specifically, the alignment of the protein sequences of glutaminase (gls) (Exhibit A in the July 31, 2007 response), one of ordinary skill in the art would clearly recognize which regions are important for the enzymatic activities of the proteins, and would be able to reduce the activities of the proteins by introducing amino acid mutations at such regions, and hence the claims are fully and adequately described. Furthermore, one of ordinary skill in the art would be able to readily ascertain expression regulatory sequence of the recited glutaminase gene on the chromosome of a coryneform bacterium based on the sequence information for glutaminase genes, and would be able to mutate or disrupt the expression regulatory sequence of the recited glutaminase gene. That is, one of ordinary skill in the art would be able to obtain a coryneform bacterium in which glutaminase activity is reduced to 0.1U/mg of cellular protein or less, by mutating or disrupting the recited glutaminase gene on the chromosome of a coryneform bacterium and/or by mutating or disrupting expression regulatory sequence of the recited glutaminase gene, based on the known sequence information for glutaminase genes, the teaching of the specification, and the knowledge and level of skill in this art. No undue experimentation is required to determine such information, and the invention is fully and adequately described, particularly in view of the knowledge in the prior art regarding the sequences.

Also, as is evidenced by the alignment of the nucleotide sequences of glutamine synthetase gene (gin) from *B. flavum* (SEQ IDNO: 3), *C. glutamicum, C. efficiens,* and *Mycobacterium tuberculosis,* gln genes are highly conserved (Exhibit B in the July 31, 2007 response). Therefore, one of ordinary skill in the art would reasonably understand that a DNA sequence which has 95% or more homology to SEQ ID NO: 3 would naturally encode a protein having glutamine synthetase activity. Thus, one of ordinary skill in the art would reasonably be able to ascertain structures of the recited homologous gene which encodes a glutamine synthetase. Also, claims 5 and 16 have been amended to restrict means for increasing glutamine synthetase activity to "by increasing the copy number of glutamine synthetase gene" or "by replacing promoter region of glutamine synthetase gene with a stronger promoter". These two means are fully supported by the present specification (see page 17, lines 15—21) Therefore, one of ordinary skill in the art is believed to reasonably understand that by increasing the copy number of the recited glutamine synthetase gene or by replacing promoter region of the recited glutamine synthetase gene with a stronger promoter, glutamine synthetase activity would be enhanced.

Finally, the Examiner has rejected the claims under 35 U.S.C. §103 as reciting subject matters that allegedly are obvious, and therefore allegedly unpatentable, over the disclosure of Nakamura et al. in view of the disclosure of Pompejus et al. and further in view of Duran et al. Again, the Examiner explains that the phrase "95% or more homologous" has not been given patentable weight. However, the teachings of the prior art when viewed in light of the ***ALL*** the claim limitations, clearly does not render the claims obvious or anticipated, singly or in combination, for the following reason.

Duran et al. teach that the glutaminase-deficient mutant strain of *Rhizobium etli* (LM16) demonstrated an increase in the intracellular glutamine level when the mutant strain was cultivated in the presence of glutamine as the carbon source (Table 1). However, this reference only teaches uptake of glutamine added to the medium by the bacterial cells, and does not show production of glutamine by the LM16 strain in the culture medium. It can be interpreted that the phenomenon that "intracellular glutamine levels are increased when glutamine is used as a carbon source" arises because glutamine which has been imported into the bacterial cells is not degraded in the absence of glutaminase. Furthermore, when succinate and glutamine were simultaneously added to the medium, intracellular glutamine levels decreased as compared to when glutamine was used as the sole carbon source. Such strain is not believed to have the ability to produce glutamine.

On the other hand, the bacterium as claimed in the present application is a coryneform bacterium which has L-glutamine-producing ability. As described in the specification of the present application (see page 6, lines 17-19), "L-glutamine-producing ability" means an ability of the bacterium to accumulate L-glutamine in a medium when the bacterium is cultivated in the medium. Such bacterium having L-glutamine-producing ability is believed not to be disclosed or suggested by Duran et al.

Furthermore, *Rhizobium* bacterium is known to have an enzyme which catalyzes the formation of 2 molecules of glutamic acid from 1 molecule of glutamine and 1 molecule of α-ketoglutarate (αKG) in the presence of NADPH. This enzyme is called GOGAT, and is also present in coryneform bacterium. GOGAT does not function in the absence of αKG.

As is seen in Table 1 of Duran et al., glutamine accumulates in the cells when glutamine is added as the sole carbon source. This is probably because when glutamine is used as the sole carbon source, αKG is not produced in the cells and GOGAT does not work. On the other hand, simultaneous addition of succinate and glutamine lead to a marked decrease in intracellular glutamine levels as compared to when glutamine is present as the sole carbon source. This is probably because GOGAT works to degrade glutamine in the presence of succinate. The reason why GOGAT works to degrade glutamine in the presence of succinate is that when succinate is added, αKG is produced via the TCA cycle. Also, because pyruvate is formed from succinate, and then acetyl CoA is formed from pyruvate, the TCA cycle can use the produced acetyl CoA. It is well known that glutamine fermentation is typically performed in the presence of glucose. During such fermentation, αKG accumulates in the fermentation broth. Therefore, a sufficient amount of αKG is present in the cells when the cells are cultivated in the presence of glucose. Also, producing αKG from glucose (C6→C5) is more efficient than producing αKG from succinate (C4→C5), because the former does not need an extra metabolic pathway. Therefore, GOGAT would be expected to have a greater contribution to the degradation of glutamine when glucose is added to the medium, as compared to when succinate is added to the medium.

In view of the above, Duran et al. clearly do not teach or suggest an increased glutamine level in a glutaminase-deficient bacterium when glucose is present in the medium. In fact, Duran

et al. actually teaches away from the claimed invention , that in the presence of glucose, glutamine levels in such bacterium decrease.

Nakamura et al. teach glutamine fermentation by a coryneform bacterium, which is typically performed in the presence of glucose in the medium. However, there is no teaching in Nakamura et al. of disabling the glutaminase gene. Pompejus et al. teach the genes encoding the glutaminase and glutamine synthetase genes from *C. glutamicum*, which applicants do not dispute. However, there is no reason, for the reasons stated above, that one of ordinary skill in the art would be motivated or have any reason to combine the teachings of these three references. Most notably, this is because, one of ordinary skill in the art would have reasonably concluded that the glutamine level in bacterial cells would ***decrease***, based on the teachings of Nakamura et al. combined with the teaching of Duran et al. and Pompejus et al., which is opposite to the effect of the claimed invention.

Thus, there is no reason or motivation to combine Nakamura et al., Duran et al., and/or Pompejus et al. for the purpose of breeding a glutamine-producing bacterium. Furthermore, through no combination of the cited references is it obvious for one of ordinary skill in the art to modify a coryneform bacterium to reduce intracellular glutaminase activity with the expectation of obtaining a bacterium with enhanced L-glutamine producing ability. This rejection should be withdrawn.

Conclusion

In the interest of brevity, Applicant does not provide all arguments that would support an appeal for each of the pending and rejected claims. However, it is respectfully submitted that this case is in immediate and clear form for allowance based on the clear errors and omissions cited above. Accordingly, an early indication via a Notice of Allowability that all claims are allowable is respectfully requested. Should any questions arise in connection with this application or should the Examiner believe that a telephone conference with the undersigned would be helpful in resolving any remaining issues pertaining to this application, the undersigned respectfully requests that he be contacted at the number indicated below.

Respectfully submitted,

By: _____

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